THE INFLUENCE OF IRON ON THE VIRULENCE OF THE PLAGUE MICROBE FOLLOWING PASSAGING OF THE CULTURE ON ARTIFICIAL NUTRIENT MEDIA

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The virulence of the plague microbe plays a great role in the pathogenesis of plague morbidity. A number of authors succeeded in establishing the presence of a direct dependency between the virulence of the plague microbe and its catalase activity (Rockenmacher, 1949; Zaplatina and Borodina, 1955).

L. A. Avanyan and N. Ye. Gubina (1960 a, b) showed the ability of the plague microbe to utilize a large quantity of iron if there was an increased content of it in meat-peptone agar. It has been established that ferrous sulfate and Mohr's salt stimulate the growth even of individual cells of the plague microbe on nutrient media. Based on the data of these authors, the presence of free ions of iron in the nutrient medium guarantees the optimal conditions for the synthesis of a number of iron containing enzymes by the plague microbes, including catalase.

S. Jackson and T. Burrows (1956), and L. A. Avanyan and N. Ye. Gubina (1960) stably increased the virulence of the plague microbe by means of numerous passages of it through the organism of white mice and guinea pigs with the simultaneous administration of ferrous salts to them.

The aim of the present work was to establish the influence on the virulence of the plague microbe of numerous passages of it on nutrient media containing ferrous salt (Mohr's salt -- $FeSO_4(NH_4)_2SO_4.H_2O$).

Work was conducted with the weakly virulent culture 138 of the plague microbe with typical cultural and morphological properties, with the exception of the ability to decompose lactose in 10 days. Plague microbe culture 138 was passaged on meat-peptone broth, pH=7.3, containing 0.08% of Mohr's salt.* Besides Mohr's salts, hemolyzed blood and guinea pig liver were added to the meat-peptone broth in various combinations. The control was meat-peptone broth without the addition of iron and other plague microbe growth stimulators. All told six variants of nutrient media were used.

The tests were carried out at a temperature of 28 and 37°, under aerobic and anaerobic conditions of incubation for a period of 48 hours for each passage. The first 20 passages on nutrient media were conducted in such a way that the cultivation on liquid nutrient medium was alternated with incubation on Hottinger agar with the addition of 0.08% Mohr's salt. The last 10 passages were conducted only on liquid nutrient media. The inoculation of plague microbe 138 cultures on liquid nutrient media and the seedings from them on agar plates were done in the same strictly determined dose.

Following the passaging of the culture on all the variants of nutrient media used by us at an incubation temperature of 37°, beginning with the 10th passage there was noted the dissociation of the plague microbe culture into the R --chromogenic, achromogenic and OS -- forms. Besides this, it was established that numerous passaging at a temperature of 37° on media containing iron, blood and guinea pig liver led to a lowering of the virulence of the plague microbe. As an example, while the initial culture of the plague microbe caused the death from plague septicemia of 4 out of 12 guinea pigs, infected intraperitoneally with doses of from 1 up to 15 billion microbial bodies, following the administration of the passaged culture all the animals remained alive.

Subsequently the passaging of plague microbe culture 138 on the stated nutrient media was conducted only at a temperature of 28°. The phenomena of dissociation were not noted morphologically.

During the study of the biochemical activity of the passaged culture, it was established that under specific conditions of passaging on artificial nutrient media, plague microbe culture 138 acquired the ability to ferment rhamnose and saccharose with the formation of acid (table 1).

As is seen from this table, following 20 passages on meat-peptone broth under anaerobic conditions of cultivation, strain 139 started to ferment rhamnose in 9 days. After 30 passages the fermentation activity in respect to rhamnose increased and the strain under study began to decompose it in 4 days. Fermentation of rhamnose was also noted after 30 passages on meat-peptone broth, containing iron and blood, under the conditions of aerobic incubation.

The fermentation activity in respect to rhamnose changed after 30 passages on meat-peptone broth with the addition of blood and liver fragments under anaerobic conditions of cultivation.

The virulent properties of the initial and passaged culture were determined on guinea pigs following their intraperitoneal infection. The results of the virulence determination after 30 passages are presented in table 2.

As is seen from table 2, during passaging on nutrient media under aerobic conditions, the addition of ferrous salt promoted a certain increase of virulence of the passaged culture. While following infection with the microbes of the initial strain the death of guinea pigs from all the tested doses comprised 33%, following 30 passages, carried out on meat-peptone broth with the addition of ferrous salt, 41% of the animals perished. After the same number of passages on meat-peptone broth, containing, in addition to ferrous salt, hemolyzed blood and guinea pig liver fragments, 50% of the animals perished.

The passaging of plague microbe strain 138 under aerobic conditions on meat-peptone broth with the addition of blood, blood and guinea pig liver fragments, but without the addition of iron, led to a lowering of virulence -- out of all the tested doses, from 0 up to 16% of the infected animals perished. All 30 passages on these same nutrient media, but with the addition of iron under aerobic conditions of incubation, did not change the virulence of the plague microbe, and in a number of cases led to its lowering. As an example, from all the infection doses tested the initial culture of P. pestis 138 caused the death of 33% of the guinea pigs. Following 30 passages, carried out under anaerobic conditions of incubation on media with iron, from 8 up to 33% of the test animals perished.

Under these same conditions of the test, following 30 passages, conducted under anaerobic conditions on meat-peptone broth without iron, but containing blood and liver fragments, a lowering of virulence was observed. It is interesting to note that passaging under anaerobic conditions on meat-peptone broth containing hemolyzed blood considerably increased the virulence of the culture from all the doses tested (58% of the guinea pigs perished).

It is possible that blood hemoglobin is necessary for the plague microbe as a source for the synthesis of hemin ferments, catalase and peroxidase, which play an active role in the oxidation-reduction processes under anaerobic conditions of incubation.

An analysis of the materials presented in the tables gives a foundation to the consideration that following numerous passages on artificial nutrient media, containing iron, under the conditions of aerobic incubation a lowering of virulence does not take place.

Conclusions

1. Following 30 successive passages on artificial nutrient media, containing increased concentrations of ferrous salt, the virulence of plague microbe culture 138, following passaging under aerobic conditions, did not change noticeably.

2. Following passaging under anaerobic conditions, in some cases a lowering of virulence was noted.

3. Incubation under anaerobic conditions on media containing guinea pig blood as the nutrient substrate promoted a certain increase in virulence.

Literature

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Table 1

Biochemical Activity of the Initial and Passaged strain 138 of P. pestis

Medium on which passaging carried out	Conditions of cultivation	XX passages											XXX passages									
		Glucose	Lactose	Rhamnose	Maltose	Mannitol	Saccharose	Denitrification	Nitrification	Glycerin	Urea	Methylene blue	Glucose	Lactose	Rhamnose	Maltose	Mannitol	Saccharose	Denitrification	Nitrification	Glycerin	Urea	Methylene blue
Meat-peptone broth (MPB)	anaerob.	K	K10d	K9d	K	K	-	-	-	K	-	-	K	K10d	K4d	K	K	-	-	-	K	-	-
	aerobic	K	K10d	-	K	K	-	-	-	K	-	-	K	K10d	-	K	K	-	-	-	K	-	-
MPB + iron	anaerob.	K	K10d	-	K	K	-	-	-	K	-	-	K	K10d	-	K	K	-	-	-	K	-	-
	aerobic	K	K10d	-	K	K	-	-	-	K	-	-	K	K10d	-	K	K	-	-	-	K	-	-
MPB + blood	anaerob.	K	K10d	-	K	K	-	-	-	K	-	-	K	K10d	-	K	K	K7d	-	-	K	-	-
	aerobic	K	K10d	-	K	K	-	-	-	K	-	-	K	K10d	-	K	K	-	-	-	K	-	-
MPB + blood + iron	anaerob.	K	K10d	-	K	K	-	-	-	K	-	-	K	K10d	-	K	K	-	-	-	K	-	-
	aerobic	K	K10d	-	K	K	-	-	-	K	-	-	K	K10d	K4d	K	K	-	-	-	K	-	-
MPB + blood + liver	anaerob.	K	K10d	-	K	K	-	-	-	K	-	-	K	K10d	-	K	K	K7d	-	-	K	-	-
	aerobic	K	K10d	-	K	K	-	-	-	K	-	-	K	K10d	-	K	K	-	-	-	K	-	-
MPB + blood + liver + iron	anaerob.	K	K10d	-	K	K	-	-	-	K	-	-	K	K10d	-	K	K	-	-	-	K	-	-
	aerobic	K	K10d	-	K	K	-	-	-	K	-	-	K	K10d	-	K	K	-	-	-	K	-	-
Initial strain	aerobic	K	K10d	-	K	K	-	-	-	K	-	-	K	K10d	-	K	K	-	-	-	K	-	-

Legend: K -- acid; the number following the letter K designates on which day the acid is formed;
- negative result.

Table 2

Virulence of the plague microbe (strain 138) for guinea pigs following 30 passages on nutrient media with iron.

Conditions of cultivation	Aerobic, temperature 28°					Anaerobic, temperature 28°				
Dose of culture / Nutrient media	1 bil. m.b.	5 bil. m.b.	15 bil. m.b.	Number of guinea pigs perished	Percentage lost from all doses	1 bil. m.b.	5 bil. m.b.	15 bil. m.b.	Number of guinea pigs perished	Percentage lost from all doses
Meat-peptone broth	4/0	4/1	4/3	12/4	33	4/0	4/2	4/3	12/5	41
MPB + iron	4/1	4/2	4/2	12/5	41	4/0	4/1	4/0	12/1	8
MPB + blood	4/0	4/0	4/0	12/0	0	4/1	4/3	4/3	12/7	58
MPB + blood + iron	4/0	4/4	4/1	12/5	41	4/1	4/2	4/1	12/4	33
MPB + blood + liver	4/1	4/1	4/0	12/2	16*	4/0	4/2	4/0	12/2	16
MPB + blood + liver + iron	4/1	4/1	4/4	12/6	50	4/1	4/2	4/1	12/4	33
Control. Initial unpassaged culture 138	4/2	4/0	4/2	12/4	33					

Legend: * Result obtained during determination of virulence following 20th passage.
MPB - Meat-peptone broth; m.b. - microbial bodies; numerator - number of animals in the group; denominator - number of animals lost.